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Bartonella australis sp. nov. from Kangaroos, Australia

To the Editor: During April–May 1999, 3 *Bartonella* isolates (AUST/NH1, AUST/NH2, AUST/NH3) were cultivated and established from the blood of 5 *Macropus giganteus* gray kangaroos from central coastal Queensland, Australia. We used multigene sequencing to evaluate whether these *Bartonella* isolates fulfill the minimum requirements for classification as a new species.

DNA from each *Bartonella* isolate was extracted by using the QIAamp tissue kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Partial PCR amplification and sequencing of the genes encoding the 16S rDNA (*rrs*), citrate synthase (*gltA*), β-subunit of the RNA polymerase (*rpoB*), and cell division protein (*ftsZ*), as well as for the 16S–23S rDNA intergenic spacer (ITS) were attempted by using previously described primers and conditions (*1*). *Bartonella* sp. isolates AUST/NH1 to AUST/NH3 exhibited identical sequences for all 4 genes and the spacer studied, and isolate AUST/NH1 was selected as type strain among kangaroo isolates. Similarity rates between strain Aust/NH1 and validated *Bartonella* species (Appendix Table) ranged from 84.7% to 91.6%, from 97.5% to 98.5%, from 79.6% to 87.2%, from 85.4% to 95.0%, and from 83.5% to 87.1% for the ITS and *rrs*, *gltA*, *rpoB,* and *ftsZ* genes, respectively. Therefore, for each of these 4 genes or the spacer, strain AUST/NH1 exhibited similarity rates with all other species lower than the cutoffs published to classify *Bartonella* isolates within a validated species (*1*). It may thus be regarded as a new species.

To estimate the genomic G+C content of strain AUST/NH1, we amplified and sequenced its *ftsY* gene as described (*2*) by using the BartftsyF (5′-ATGACAAAAYCYTTTATMAA-3′) and BartftsyR (5′-TCATGAGTGTCTTCCTGC-3′) primers. The *ftsY* G+C content was 37.7%;

the calculated genomic G+C content was 39.51%. The *ftsY* sequence was deposited in GenBank under accession no. DQ538398.

The phylogenetic relationships among the studied bartonellae were inferred from sequence alignments of each gene and from concatenated gene sequences by using the maximum parsimony and neighbor-joining methods within the MEGA version 2.1 software package (*3*) and the maximum-likelihood method within the PHYLIP software package (*4*). Using *rrs*, *gltA*, and *rpoB* sequences, the phylogenetic position of strain AUST/NH1 was supported by bootstrap values <70%. In contrast, by using the ITS, *ftsZ,* and concatenated sequences, strain AUST/NH1 clustered with a group of *B. tribocorum*, *B. grahamii*, and *B. elizabethae*, with elevated bootstrap values according to the 3 analysis methods (Figure).

The *Bartonella* strains we describe are the first, to our knowledge, obtained from kangaroos and, more generally, from marsupials. Before this study, the only 2 *Bartonella* species found in Australia were *B. henselae* (*5*) and *B. quintana* (*6*). We demonstrated that strain AUST/NH1 was reliably associated with a well-established cluster, including the rodent-associated *B. elizabethae*, *B. grahamii*, and *B. tribocorum* (*7*). Therefore, we are confident that the phylogenic position of the new *Bartonella,* which was similar according to 3 analysis methods and supported by high bootstrap values, is reliable. Although *B. grahamii* (*8*) and *B. elizabethae* (*9*), members of the same phylogenetic cluster as strain AUST/NH1, cause human infections, the pathogenicity of *B. tribocorum* is as yet unknown. Its pathogenicity should therefore be investigated, especially for persons who come in contact with kangaroos.

B. australis is a facultative intracellular gram-negative bacterium. It grows on Columbia agar with 5% sheep blood at 32°C to 37°C in a moist atmosphere containing 5% CO_2. A primary culture was obtained after 7 days, and subculture was obtained after 4 days under the same conditions. Colonies are homogeneous, smooth, round, and gray-white. The 3 strains tested were oxidase negative, catalase negative, and nonmotile. Pathogenicity for humans is, as yet, unknown.

The type strain is strain AUST/NH1. The new species is distinguished from other *Bartonella* species by its 16S rRNA, *gltA*, *rpoB*, *ftsZ* gene sequences, as well as its 16S–23S rRNA ITS sequence. The estimated G+C content is 38%. The type strain exhibits a specific serotype (*10*) and was susceptible to amoxicillin, ceftriaxone, imipenem, erythromycin,

clarithromycin, ofloxacin, ciprofloxacin, rifampin, and tetracycline (unpub. data). The type strain AUST/NH1 has been deposited in the Collection of the World Health Organization Collaborative Center for Rickettsioses, Borrelioses and Tick-borne Infections (CSUR), Marseille, France, under reference CSUR B1; in the Collection de l'Institut Pasteur (CIP) under reference CIP 108978T; and in the Culture Collection of the University of Göteborg (CCUG), Sweden, under reference CCUG 51999. The strains AUST/NH2 and AUST/NH3 have been deposited in CSUR under references CSUR B2 and CSUR B3, in the CIP under references CIP 108980 and CIP 108979, and in CCUG under references CCUG 52000 and CCUG 52001, respectively.

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Pierre-Edouard Fournier,* Carmel Taylor,† Jean-Marc Rolain,* Lina Barrassi,* Greg Smith,† and Didier Raoult*

*Université de la Méditerranée, Marseille, France; and †Queensland Health Scientific Services, Coopers Plains, Queensland, Australia

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Address for correspondence: Didier Raoult, Unité des Rickettsies, IFR 48, CNRS UMR 6020, Faculté de Médecine, Université de la Méditerranée, 27 Boulevard Jean Moulin, 13385 Marseille CEDEX 05, France; email: didier.raoult@medecine.univ-mrs.fr

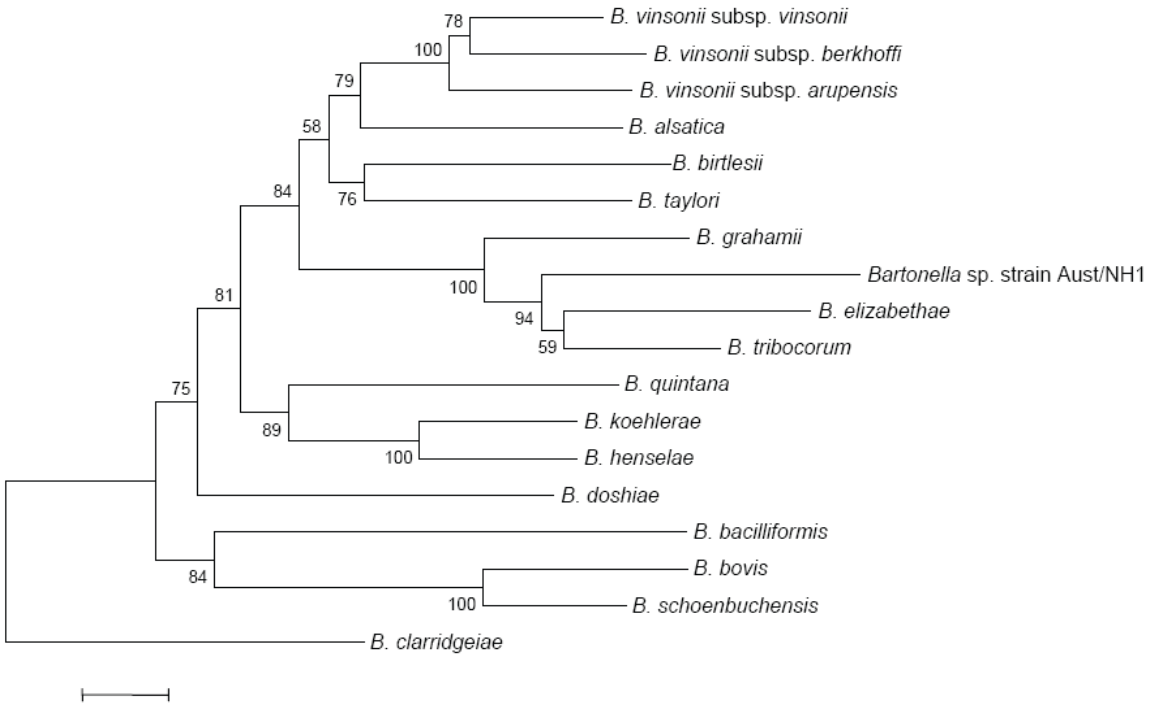

Figure. Unrooted dendrogram showing the phylogenetic position of *Bartonella* sp. strain AUST/NH1 among *Bartonella* species inferred from the comparison of concatenated sequences from the *rrs, gltA,* intergenic spacer, *rpoB,* and *ftsZ* genes by the neighbor-joining method. We included only species for which all 5 genes were available. Bootstrap values are indicated at the nodes. The scale bar indicates nucleotide sequence divergence of 0.5%.

Appendix Table. *Bartonella* spp. and sequences used to validate *Bartonella* isolates (AUST/NH1, AUST/NH2, AUST/NH3) from 5 *Macropus giganteus* gray kangaroos, Australia, 1999*

		GenBank accession no.				
Species	Strain	16S rRNA	16S-23S rRNA	*gltA*	*rpoB*	*ftsZ*
B. alsatica	IBS 382^T	AJ002139	AF312506	AF204273	AF165987	AF467763
B. bacilliformis	KC584^T	Z11683	L26364	U280276	AF165988	AF007266
B. birtlesii	IBS 325^T	AF204274	AY116640	AF204272	AF165989	AF467762
B. bovis	91–4^T	AF199502	AY116638	AF293394	AF166581	AF467761
B. capreoli	IBS 193^T	AF293389	NA	AF293392	NA	NA
B. chomelii	A828^T	AY254309	NA	AY254309	NA	NA
B. clarridgeiae	Houston-2^T	U64691	AF167989	U84386	AF165990	AF141018
B. doshiae	R18^T	Z31351	AJ269786	AF207827	AF165991	AF467754
B. elizabethae	F9251^T	L01260	L35103	U28072	AF165992	AF467760
B. grahamii	V2^T	Z31349	AJ269785	Z70016	AF165993	AF467753
B. henselae	Houston-1^T	M73229	L35101	L38987	AF171070	AF061746
B. koehlerae	C-29^T	AF076237	AF312490	AF176091	AY166580	AF467755
B. peromysci		U71322	U77057	NA	NA	NA
B. quintana	Fuller^T	M11927	L35100	Z70014	AF165994	AF061747
B. schoenbuchensis	R1^T	AJ278187	AY116639	AJ278783	AY167409	AF467765
B. talpae†		NA	NA	NA	NA	NA
B. taylorii	M6^T	Z31350	AJ269784	AF191502	AF165995	AF467756
B. tribocorum	IBS 506^T	AJ003070	AF312505	AJ005494	AF165996	AF467759
B. vinsonii subsp. *arupensis*	OK 94–513	AF214558	AF312504	AF214557	AY166582	AF467758
B. vinsonii subsp. *berkhoffii*	93-CO1	L35052	AF312503	AF143445	AF165989	AF467764
B. vinsonii subsp. *vinsonii*	Baker^T	M73230	L35102	Z70015	AF165997	AF467757
B. australis	AUST/NH1^T	DQ538394	DQ538396	DQ538395	DQ538397	DQ538399

*Superscript T, type strain; NA, not available.
†No sequence available for this species.